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PREPARATION OF SUBMICRON SOLID PARTICLE SUSPENSIONS BY SONICATION OF MULTIPHASE SYSTEMS

DESCRIPTION

CROSS-REFERENCE TO RELATED APPLICATIONS:

Not Applicable.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT:

Not Applicable.

TECHNICAL FIELD

The present invention provides a multistep process for generating submicron-sized particles of water insoluble organic compounds and more particularly a process for preparing submicron-sized particles of a pharmaceutically effective compound by sonicating a multiphase liquid.

BACKGROUND OF THE INVENTION:

Background Art:

The ability to deliver pharmaceutical medications in a water-soluble formulation is a critical concern in therapeutic drug development. The fact that poorly soluble drugs can pose difficulties in this area has resulted in the need for new technologies that can address this obstacle. One solution to this problem is the production of extremely small particles of the insoluble drug candidate and the creation of a microparticulate suspension. In this way, drugs

that were previously unable to be formulated in an aqueous based system can be made suitable for parenteral administration. Suitability for parental administration includes small particle size (<7μm), low toxicity (as from toxic formulation components or residual solvents), and bioavailability of the drug particles after administration.

One approach utilized with the anesthetic, propofol (2,6 diisopropylphenol) as an example, involves incorporating the pharmacological agent in a vegetable oil emulsion to enable intravenous administration. See, e.g., U.S. Pat. Nos. 4,056,635; 4,452,817 and 4,798,846, all to Glen et al. Such emulsions, however, tend to be unstable given the predominance of the oil phase and the absence of antimicrobial agents. In other instances, even where the pharmacological agent is successfully incorporated into an oil-free formulation, particles containing the pharmacological agent may cause irritation at the site of delivery because of their size or form.

A variety of approaches have been explored for formulating a substantially water-insoluble pharmacologically active agent for *in vivo* delivery. One approach is directed to the production of suspended particles coated with protein. U.S. Patent 5,916,596, issued to Desai et al., discloses the application of high shear to a mixture of an organic phase having a pharmacologically active agent dispersed therein and an aqueous medium containing a biocompatible polymer. The mixture is sheared in a high-pressure homogenizer at a pressure in the range of from about 3,000 to 30,000 psi. The claims explicitly provide that the mixture must contain substantially no surfactants because the combined use of a surfactant with a protein results in the formation of large, needle-like crystalline particles that increase in size during storage. *See* columns 17-18, example 4. Example 2 discloses that crude emulsion may be sonicated to produce nanoparticles ranging from 350-420 nanometers.

U.S. Patent No. 5,560,933, issued to Soon-Shiong et al., discloses the formation of a polymeric shell around the water-insoluble drug for *in vivo* delivery. The method discloses the application of sonication to a mixture comprising a polymer-containing aqueous medium and a dispersing agent having a substantially water-insoluble drug dispersed therein. In this reference, sonication is used to drive the formation of disulfide bonds in the polymer, causing it to crosslink so as to produce a polymeric shell around the drug. Sonication is conducted for a time sufficient for the disulfide bonds to form.

In U.S. Patent No. 5,665,383, Grinstaff et al. discloses the application of ultrasound to a single-phase B i.e., an aqueous medium -- to encapsulate an immunostimulating agent within a polymeric shell for *in vivo* delivery. The ultrasound promotes crosslinking of the encapsulating agent by disulfide bonds to form the shell.

Another approach to preparing a water-insoluble drug for *in vivo* delivery centers on reducing the size of the particles that deliver the drug. In one such series of patents, which

include U.S. Patents 6,228,399; 6,086,376; 5,922,355; and 5,660,858, Parikh *et al.* discloses that sonication may be used to prepare microparticles of the water-insoluble compound. Of these patents, U.S. Patent No. 5,922,355 discloses an improvement to a method that uses sonication for making the smaller particles. The improvement comprises mixing an active pharmacological agent with a phospholipid and surfactants in a single-phase aqueous system and applying energy to the system to produce the smaller particles.

U.S. Patent No. 5,091,188, issued to Haynes, also discloses reducing the size of particles of a pharmacologically active water-insoluble drug and employing a lipid coating on the particles to confer a solid form. The patent is directed to a pharmaceutical composition consisting essentially of an aqueous suspension of solid particles of the drug having a diameter of about 0.05 to about 10 microns. The lipid coating affixed to the surface of the particles contributes to their solid form. The composition is produced by adding the drug to water and then reducing the particle size within the aqueous suspension. Example 6 of this reference discloses the use of a pharmacologically acceptable oil which is selected for its inability to dissolve the crystalline drug. *See* column 16, lines 8-12.

Still another approach for preparing microparticles of a pharmacological agent focuses on the use of phase inversion principles. U.S. Patent Nos. 6,235,224 B1 and 6,143,211, both issued to Mathiowitz et al., disclose the use of phase inversion phenomena to precipitate microencapsulated microparticles. The method includes mixing a polymer and a drug with a solvent. This mixture is introduced into an effective amount of a miscible nonsolvent, thereby causing spontaneous formation of the microencapsulated product.

Microprecipitation is another technology used to prepare dispersions of a nanoparticulate pharmaceutical agent. See, e.g., U.S. Patent Nos. 5,766,635; 5,665,331; and 5,662,883. This technology involves dissolving a pharmaceutical in an aqueous base that is then neutralized to form a dispersion.

In yet another approach, such as that disclosed in U.S. Patent No. 5,766,635, issued to Spenlenhauer et al., nanoparticles have been prepared by dissolving a poly(ethylene) oxide and/or poly(propylene) oxide in an organic solvent, mixing the organic solution so formed with an aqueous solution to cause nanoparticles to precipitate out of solution, and microfluidizing the precipitated solution without the use of surfactants.

Because of the difficulties posed by poorly soluble drugs in drug therapy, the need for new technologies continues to expand for addressing these problems.

SUMMARY OF THE INVENTION:

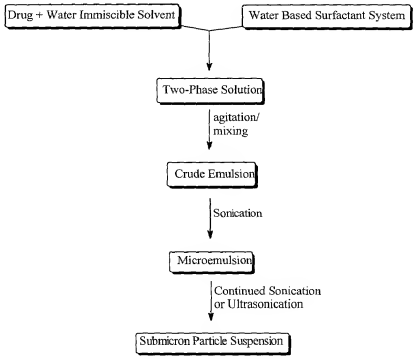
The present invention provides a method for preparing submicron-sized particles. The method includes the steps of: (1) providing a multiphase system having an organic phase and an

aqueous phase, the organic phase having a pharmaceutically effective compound therein; and (2) sonicating the system to evaporate a portion of the organic phase to cause precipitation of the compound in the aqueous phase and having an average effective particle size of less than about 2 μm.

The present invention further provides a process for preparing an aqueous suspension of submicron sized particles. The process includes the steps of: (1) providing an organic phase of a pharmacologically active compound dissolved in a water immiscible solvent, (2) providing an aqueous phase, (3) combining the organic phase with the aqueous phase, and (3) sonicating the emulsion to cause precipitation of the compound as a suspension of particles in the aqueous phase wherein the aqueous phase is essentially free of the water immiscible solvent.

BRIEF DESCRIPTION OF THE DRAWINGS:

Figure: 1



DETAILED DESCRIPTION OF THE INVENTION:

While this invention is susceptible of embodiment in many different forms, there is shown in the drawings and will herein be described in detail a preferred embodiment of the invention with the understanding that the present disclosure is to be considered as an exemplification of the principles of the invention and is not intended to limit the broad aspect of the invention to the embodiment illustrated.

The present invention provides a process for preparing submicron-sized particle suspensions. The process comprises the steps of: (1) providing a multiphase system having an organic phase and an aqueous phase, the organic phase having a pharmaceutically effective compound therein; and (2) sonicating the system to evaporate a portion of the organic phase to cause precipitation of the compound in the aqueous phase and having an average effective particle size of less than about 2μm. The step of providing a multiphase system includes the steps of: (1) mixing a water immiscible solvent (oil phase) with the pharmaceutically effective compound to define an organic solution, (2) preparing an aqueous based solution with one or more surface active compounds, and (3) mixing the organic solution with the aqueous solution to form the multiphase system. The multiphase system can be agitated or mixed to form a crude emulsion. The crude emulsion will have oil droplets in the water of a size of approximately less than 1 μm in diameter. The crude emulsion is sonicated to define a microemulsion and eventually to define a submicron particle suspension. (See Figure 1 above).

What is meant by the term "multiphase system" is an emulsion having at least one organic phase and at least one aqueous phase and in a preferred form of the invention is an oil in water (O/W) emulsion where the water phase forms the continuous phase and the oil phase forms the dispersed phase. The ratio by weights of the organic phase to the aqueous phase is from about 1:99 to about 40:60, more preferably from about 2:98 to about 30:70 or any range or combination of ranges therein. The present invention further contemplates utilizing reverse emulsions or water in oil emulsion (W/O) where the oil phase forms the continuous phase and water the dispersed phase. The present invention further contemplates utilizing emulsions having more than two phases such as an oil in water in oil emulsion or O/W/O.

What is meant by the term "pharmaceutically effective compound" is any compound that has a therapeutic effect and more particularly to such compounds that are insoluble or slightly soluble in water (those having a solubility of less than 10 mg/ml). Such compounds can be found in the Physicians' Desk Reference (PDR), which is incorporated herein by reference. Particularly suitable pharmaceutically active compounds includes, but is not limited to, antihyperlipidemics; antimicrobials, e.g., antibacterials such as sulfadiazine, antifungals such as itraconazole; non-steroidal anti-inflammatory drugs, e.g., indomethacin; antihypercholesteremic agents, e.g., probucol; and steroidal compounds, e.g., dexamethasone; immunosuppresants, e.g., cyclosporin A, tacrolimus, and mycophenolate mofetil. Or the organic compound might be from the group used as adjuvants or excipients in pharmaceutical preparations and cosmetics, such as, but not limited to, preservatives, e.g., propylparaben.

The pharmaceutically effective compound can be present in a concentration to the extent it is soluble in the organic phase. In a preferred form of the invention the pharmaceutically effective compound can be present in an amount from less than 1% to about 40%, more

preferably from about 1% to about 25%, and most preferably from about 1% to about 10% by weight of the organic phase or any range or combination of ranges therein.

What is meant by the term "water immiscible solvent" are those solvents that form an interfacial meniscus when combined with an aqueous solution in a 1:1 ratio (o/w). In a preferred form of the invention the water immiscible solvent (oil phase) will have a vapor pressure higher than that of water when both the solvent and water are measured at room temperature. Suitable water immiscible solvents include, but are not limited to, substituted or unsubstituted, linear, branched or cyclic alkanes with a carbon number of 5 or higher, substituted or unsubstituted, linear, branched or cyclic alkenes with a carbon number of 5 or higher, substituted or unsubstituted, linear, branched or cyclic alkynes with a carbon number of 5 or higher; aromatic hydrocarbons completely or partially halogenated hydrocarbons, ethers, esters, ketones, mono-, di- or tri-glycerides, native oils, alcohols, aldehydes, acids, amines, linear or cyclic silicones, hexamethyldisiloxane, or any combination of these solvents. Halogenated solvents include, but are not limited to carbon tetrachloride, methylene chloride, chloroform, tetrachloroethylene, trichloroethylene, trichloroethane, hydrofluorocarbons, chlorinated benzene (mono, di, tri), trichlorofluoromethane. Particularly suitable solvents are methylene chloride, chloroform, diethyl ether, toluene, xylene and ethyl acetate.

What is meant by the term "surface active compounds" are compounds such as an anionic surfactant, a cationic surfactant, a nonionic surfactant or a biological surface active molecule. The surface-active compound can be added to the organic phase, the aqueous phase or to both the organic phase and the aqueous phase. The surface active compound should be present in an amount by weight of the aqueous phase or the organic phase, whatever the case may be, from less than about 1% to about 30%, more preferably from about 1% to about 20% or any range or combination of ranges therein.

Suitable anionic surfactants include but are not limited to: potassium laurate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl inosine, phosphatidylserine, phosphatidic acid and their salts, glyceryl esters, sodium carboxymethylcellulose, cholic acid and other bile acids (e.g., cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid) and salts thereof (e.g., sodium deoxycholate, etc.).

Suitable cationic surfactants include but are not limited to quaternary ammonium compounds, such as benzalkonium chloride, cetyltrimethylammonium bromide, lauryldimethylbenzylammonium chloride, acyl carnitine hydrochlorides, or alkyl pyridinium halides. As anionic surfactants, phospholipids may be used. Suitable phospholipids include, for example, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidyl

inositol, phosphatidylglycerol, phosphatidic acid, lysophospholipids, egg or soybean phospholipid or a combination thereof. The phospholipid may be salted or desalted, hydrogenated or partially hydrogenated or natural, semisynthetic or synthetic.

Suitable nonionic surfactants include: polyoxyethylene fatty alcohol ethers (Macrogol and Brij), polyoxyethylene sorbitan fatty acid esters (Polysorbates), polyoxyethylene fatty acid esters (Myrj), sorbitan esters (Span), glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers (poloxomers), polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides including starch and starch derivatives such as hydroxyethylstarch (HES), polyvinyl alcohol, and polyvinylpyrrolidone. In a preferred form of the invention the nonionic surfactant is a polyoxyethylene and polyoxypropylene copolymer and preferably a block copolymer of propylene glycol and ethylene glycol. Such polymers are sold under the tradename POLOXAMER also sometimes referred to as PLURONIC®, and sold by several suppliers including Spectrum Chemical and Ruger. Among polyoxyethylene fatty acid esters is included those having short alkyl chains. One example of such a surfactant is SOLUTOL® HS 15, polyethylene-660-hydroxystearate, manufactured by BASF Aktiengesellschaft.

Surface-active biological molecules include such molecules as albumin, casein, heparin, hirudin or other appropriate proteins.

Sonicating

The step of sonicating can be carried out with any suitable sonication device (e.g., Branson Model S-450A or Cole-Parmer 500/750 Watt Model). Such devices are well known in the industry. Typically the sonication device has a sonication horn or probe that is inserted into the multiphase system of interest to emit sonic energy into the solution. The sonicating device, in a preferred form of the invention, is operated at a frequency of from about 1 kHz to about 90 kHz and more preferably from about 20 kHz to about 40 kHz or any range or combination of ranges therein. The probe sizes can vary and preferably is in distinct sizes such as ½ inch or ¼ inch or the like. It may also be desirable to cool the solution during sonication to temperatures below room temperature. It may also be desirable to employ other mixing devices such as homogenizers, blenders or other stirring devices to assist in the process.

Exposing the emulsion droplets to shear energy can reduce the droplet sizes. Sonication provides a source of shear energy that effectively reduces the diameters of the emulsion droplets. Shear from sonication results from the compression and rarefaction of the propagation medium of the sound waves. In pure liquids this oscillation between compression and

rarefaction is sufficiently energetic to cause cavitation, which is the tearing of the liquid to cause bubble formation. In an emulsion, the analogous process results in tearing the emulsified liquid particles into smaller particles. Cavitation and the warming of the emulsion during sonication also appear to effect removal of the water immiscible solvent. As the solvent is removed the solubility of the water-insoluble compound in the emulsion decreases, eventually allowing precipitation of the compound. Under appropriate conditions, the precipitation of the insoluble compound occurs in a manner which retains the original particle size of the sonicated emulsion.

The sonicating step is effective to remove nearly all solvent in the system to provide a particle suspension essentially free of the organic phase.

The present invention further contemplates additional processing of the resulting dispersion including removal of any residual solvent that may exist by means such as evaporation by the addition of heat or under reduced pressure, or through diafiltration. The solvent-free suspension can then be filtered through an appropriate 0.2 μm filter, resulting in a sterile suspension. This suspension is then amenable to further processing including freezing or lyophilization.

The particles of the pharmaceutically effective compound should be less than about 2 μm in diameter as determined by light scattering (HORIBA) or microscopic measurements. More preferably the particles should be less than about 1 μm, even more preferably less than about 400 nm and even more preferably less than about 200 nm and most preferably less than about 100 nm or any range or combination of ranges therein.

The particles have a generally spherical shape. Further, in a preferred form of the invention the particles will be amorphous. What is meant by amorphous is an x-ray crystal study of the particles shows virtual absence of x-ray peaks. See example 8 and accompanying figure below.

In another preferred form of the invention the aqueous phase includes a combination of a non-phospholipid containing surface active compound (selected from the group of surface active compounds set forth above) and a phospholipid containing compound selected from the group set forth above. The ratio by weight of phospholipid to the non-phospholipid surface active compound should be within the range of from about 1:99 to about 99:1, and more preferably from about 75:25 to 50:50 or any range or combination ranges therein.

EXAMPLES:

Example 1: Preparation of a 0.5% itraconazole suspension using a 1:10 ratio of O/W

A 5% lecithin/glycocholate surfactant solution was prepared (100mL) and combined with 10 mL of a chloroform solution containing itraconazole (0.5 grams). The resulting mixture was manually shaken to generate a crude emulsion and set in an ice bath to chill. After cooling

for 5 minutes the emulsion was sonicated every other minute for 10 minutes (5 minutes total sonication time at 40% power using a ½" probe at 20kHz) and then rotovapped at ~120 Torr (no heat) to remove the chloroform. The resulting solid particle dispersion was analyzed by light scattering detection (HORIBA) which revealed particles having a mean diameter of 97.78 nm.

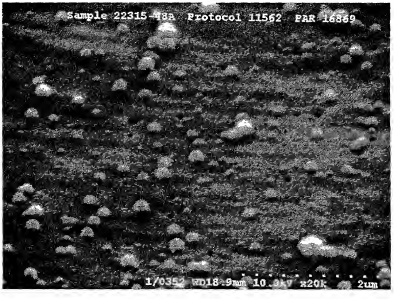
Example 2: Preparation of a 1.0% itraconazole suspension using a 1:5 ratio of O/W

A 5% lecithin/glycocholate surfactant solution was prepared (50 mL) and combined with 5 mL of a chloroform solution containing itraconazole (0.5 grams). The resulting mixture was manually shaken to generate a crude emulsion and set in an ice bath to chill. After cooling for 5 minutes the emulsion was sonicated every other minute for 10 minutes (5 minutes total sonication time) and then rotovapped at ~100 Torr (no heat) to remove the chloroform. The resulting solid particle dispersion was analyzed by light scattering detection (HORIBA) which revealed particles having a mean diameter of 135 nm.

Example 3: The process described in example 1 was repeated with the resulting particles having a mean diameter of 139 nm. This suspension was further analyzed by scanning electron microscopy to reveal solid spherical particles less than 200 nm in size. See Figure 2 for the SEM photograph.

SEM Pictures of the Itraconazole Particles:

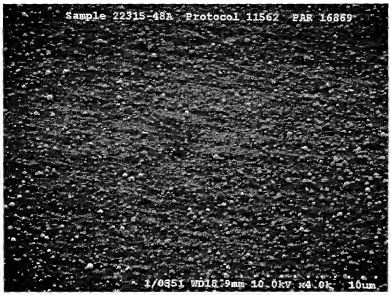
Figure 2: High magnification of itraconazole particles.



The above picture reveals the spherical nature of the particles produced. The sample was prepared by filtration of a small portion of the suspension through a 80 nm filter and using

standard SEM sample preparation techniques. Analysis of particles produced by this process revealed the particles to be completely amorphous as determined by x-ray powder diffraction.

Figure 3: Low magnification of itraconazole particles produced in example 3.



Example 4: Preparation of a 1.0% itraconazole suspension using a 2:5 ratio of O/W

A 5% lecithin/sodium glycocholate solution was prepared (50 mL) and combined with 20 mL of chloroform containing itraconazole (0.5 grams). The resulting mixture was manually shaken to generate a crude emulsion and set in ice bath to chill. After cooling for 5 minutes, the emulsion was sonicated every other minute for 8 minutes and then sonicated for 30 seconds (giving 4 minutes and 30 seconds of total sonication time) using a 1/2" probe at 40% amplitude. The sonicated dispersion was solvent evaporated at ~100 Torr (no heat) to remove chloroform. 10 mL of the final solution was filtered through a 0.2 micron filter. Both filtered and unfiltered solid particle dispersions were analyzed by light scattering detection (HORIBA), which revealed particles having a mean diameter of 110 nm and 106 nm respectively.

Example 5: A 5% lecithin/sodium glycocholate solution was prepared (50 mL) and combined with 10 mL of methylene chloride containing itraconazole (0.5 grams). The resulting mixture was manually shaken to generate a crude emulsion and set in an ice bath to chill. After cooling for 5 minutes, the emulsion was sonicated every other minute for 6 minutes (giving 3 minutes of total sonication time) using a 1/2" probe at 40% amplitude. The sonicated dispersion was solvent evaporated at ~100 Torr (no heat) to remove methylene chloride. The resulting solid particle dispersion was analyzed by light scattering detection (HORIBA), which revealed particles having a mean diameter of 144 nm

Example 6: A 5% lecithin/sodium glycocholate solution was prepared (50 mL) and combined with 5 mL of methylene chloride containing itraconazole (0.5 grams). The resulting mixture was manually shaken to generate a crude emulsion and set in ice bath to chill. After cooling for 5 minutes the emulsion was sonicated every other 30 seconds for 6 minutes (giving 3 minutes of total sonication time) using a 1/4" probe at 20% amplitude. The sonicated solution was evaporated using rotavapor at ~100 Torr (no heat) to remove methylene chloride. Resulting solid particle dispersions was analyzed by light scattering detection (HORIBA), which revealed particles having a mean diameter of 109 nm.

Example 7: Determination of solid particle size and morphology directly after sonication

The process described in example 6 was repeated except that no solvent removal was performed after sonication. Instead the sample was submitted for particle size determination by HORIBA analysis and scanning electron microscopy. HORIBA results indicated a mean particle diameter of 156 nm. The SEM pictures revealed solid spherical particles under 200 nm in size.

Example 8: Determination of Amorphous Nature of Drug Particles

A crude itraconazole emulsion was prepared by combining 50 mL of a surfactant solution (2.2% lecithin, 0.5% sodium glycocholate, 1.0% polyvinylpyrrolidone) with 5 mL of a methylene chloride solution containing 0.5 grams of itraconazole. The mixture was then manually shaken to disperse the oil droplets into the surfactant matrix.

The crude emulsion was sonicated every other 30 seconds for 6 minutes using 1/4" probe at 20% amplitude and 20 kHz (temperature ~5°C using an ice bath). The sonicated solution was then rotovapped under house vacuum (100torr) for 15-20 minutes followed by 10 minutes under a high vacuum (<20 Torr). Part of the solution was stored at -70 degrees celsius for about an hour, and subsequently lyophilized (>48 hours). Particle size of the remaining suspension was determined to be 168 nm by light scattering analysis (HORIBA). Inspection of the freeze-dried nanoparticles after lyophilization by visible light microscopy did not reveal any crystals present. Spherical particle halos were barely observable indicating that the itraconazole nanoparticles were intact.

The lyophilized itraconazole nanoparticles were assessed by X-ray powder diffraction and determined to be completely amorphous (virtual absence of x-ray peaks). In the raw material scan (the lower curve in Figure 4) many peaks are observable revealing the crystalline nature of the compound in its original state.

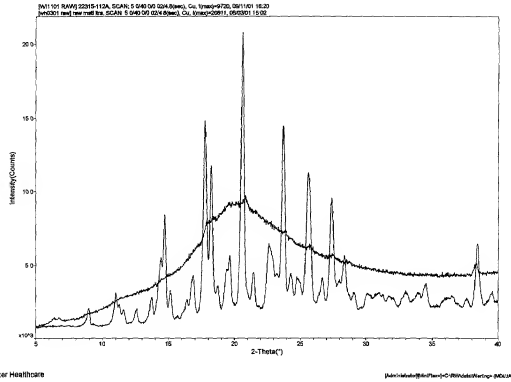


Figure 4: X-ray powder diffraction spectrum of the itraconazole nanoparticles (no peaks) and the itraconazole raw material (multiple peaks indicating crystallinity).

While the specific embodiments have been illustrated and described, numerous modifications come to mind without significantly departing from the spirit of the invention and the scope of protection is only limited by the scope of the accompanying Claims.